OVARIAN INHIBITION BY A SINUS-GLAND PRINCIPLE IN THE FIDDLER CRAB

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The action of the sinus gland in inhibiting ovarian development was first demonstrated by Panouse (1943, 1944, 1946) working with females of the shrimp, *Leander serratus*. In these animals, amputation of both eyestalks or bilateral removal of the sinus glands resulted in a great acceleration of ovarian growth, maturation of the oöcytes, and even laying of mature eggs, during a period when these structures are normally quiescent or just beginning the normal growth phase. Implantation of sinus glands into abdomens of destalked animals resulted in an inhibition of ovarian development. Similar results following eyestalk removal were obtained with the crayfish *Cambarus immunis* by Brown and Jones (1947).

The following experiments were performed upon the fiddler crab, *Uca pugilator*, to ascertain whether this phenomenon of ovarian inhibition by a blood-borne principle from the sinus glands also obtained in the division, Brachyura, of the Crustacea.

Materials and Methods

The animals used in the experiments were females of *Uca pugilator* collected near Woods Hole, Massachusetts, on July 10, 1948. The carapace widths ranged from 15 to 20 mm. at the widest point. They were kept in the laboratory at room temperature (about 25° C.) in individual containers each holding sea water to a depth of a quarter of an inch. The water was changed daily. The animals were not fed during the course of the investigation.

Removal of eyestalks was accomplished by amputation at their bases and the wounds were allowed to close spontaneously by clotting of the blood which welled slowly from them.

Sinus glands were obtained from donor animals for the purpose of implanting according to the following procedure. Eyestalks were removed as above, placed in sea water, and the contents of the eyestalks exposed by a dorsal splitting of the chitinous sheath. The sinus gland, a discrete bluish organ, was dissected free of surrounding tissue and drawn into the lumen of a 25 gauge needle by means of a tuberculin syringe. The gland, plus a minute quantity of sea water, was injected into the ventral hemocoele of the recipient animal's abdomen. The chitinous membrane of this region of the body is transparent and thus it is possible to see the actual extrusion of the contents of the needle. The dissections and implantations were accomplished with the aid of a dissecting microscope.

[1] This investigation was supported by a research grant from the graduate school of Northwestern University.

In order to observe the influences of the above procedures, experimental and normal control animals were autopsied as they died, or were sacrificed for dissection at six-day intervals. The carapace and hypodermis were removed, and the ovary, an H-shaped organ lying over the hepatopancreas and just below the hypodermis, was dissected out in sea water and placed in a tared watchglass for weighing. Excess moisture was removed with filter paper, and fresh weights were taken.

Results

On July 11, 1948, ten normal animals were sacrificed and the ovaries removed. Eight of these ovaries were found to be in an immature state; the oöcytes were very small and the organs as a whole were slender and of a light yellow-pink color. The other two ovaries contained somewhat larger oöcytes and the color of the organ was a deep shade of pink. The average of the ten ovarian weights was 12.6 mg., the extremes being 6.8 mg. and 18.8 mg. respectively

On July 12, 120 animals of nearly uniform size were selected and divided into three lots. Eyestalks were removed from two lots of forty of them. Two days later, one sinus gland was implanted into each of one lot of forty of the eyestalkless animals using the technique described earlier. The implants were then repeated every fifth day for the duration of the experiment.

Table I

Number of specimens, and ranges and averages of ovarian fresh weights in milligrams

Days	Destalked			Destalked, receiving sinus gland implants			Controls		
	No. spec.	Range	Av.	No. spec.	Range	Av.	No. spec.	Range	Av.
1–6	6	11.8–46.2	24.0	14	7.2–45.2	20.6	6	9.2–16.2	13.5
7–12	4	17.9–61.2	32.6	10	6.4–67.6	24.7	4	6.6–13.1	10.1
13–18	5	32.6–54.9	42.2	4	12.5–35.8	21.0	4	4.2–34.2	18.4
19–24	11	23.0–165.6	54.7	5	8.8–32.3	19.5	11	5.8–23.6	13.9
25–30	6	33.8–160.4	66.4	4	15.8–22.3	17.9	6	4.4–19.4	11.0

In Table I are summarized the ovarian weights of the three groups of animals (destalked, destalked and receiving sinus gland implants, and normal controls) which died or were sacrificed during five succeeding six-day periods. Each value obtained represents the average of data from 4 to 14 animals. It will be observed from Table I that over the thirty-day period the average ovarian fresh weights of the destalked animals increased approximately linearly with time from an original 12.6 mg. to 66.4 mg., a more than five-fold increase. The ovarian weights of destalked animals receiving sinus-gland implants showed an initial rise, with an approximate level being maintained at values somewhat higher (a total average of 7 mg.) than those of the control animals.

Other changes observed in the ovaries of the destalked animals, in addition to the gross size alteration, were gradual increases in oöcyte diameter and a shift of their color from the previously mentioned pinkish-yellow to a deep purple-red. The color change became most striking as the ovary attained a weight of approximately 15–18 mg.

In general, the color and size of oöcytes of the destalked animals receiving sinus-gland implants were found to lie somewhere between the extremes offered by the destalked and control animals.

During the course of the experiment, only 7 of the 31 control animals were found at autopsy to have ovaries in the apparently mature condition typical of the destalked ones. On the other hand, after the first six-day period, in no case did any of the latter group contain oöcytes presenting an immature appearance, either in size or in color.

A B

FIGURE 1. Ovaries of two fiddler crabs removed September 1, 1948: A, from an animal destalked one month earlier; B, from a normal animal.

Figure 1 is a photograph of two ovaries removed from animals of the same carapace width—17 mm. The animal from which ovary A was removed was destalked on July 30, 1948, and sacrificed for dissection on September 1, 1948. The animal from which ovary B was removed was a normal control maintained under identical laboratory conditions during the same period and sacrificed on the same day. These two organs are typical of those removed from destalked and normal animals, respectively, during the course of the experiment. The approximate wet weights are: ovary A, 80 mg.; ovary B, 10 mg.

It is also of interest to note that during the time that the investigation was in progress, five females which had been deprived of eyestalks and received no sinus-gland tissue laid mature eggs, and one female, also eyestalkless, which had received one sinus gland implant, did likewise. In none of these cases were the

eggs fastened to the pleopods of the animal as normally occurs. No eggs were laid by any of the control animals.

Discussion

It seems apparent from the foregoing results that the sinus gland in Uca, as in Leander and Cambarus, is the source of an ovary-inhibiting principle which, when absent, allows for a period of ovarian growth and development even at a time when no such gonadal activity would otherwise be manifested.

Implantation of the quantity of sinus-gland tissue utilized in this work into the abdomens of destalked animals tends to suppress the gonadal growth, but allows the ovary to be maintained at a stage somewhat more mature than that characteristic of the normal animals in possession of both sinus glands.

There is no indication from these experiments whether the principle from the sinus glands inhibits the ovary directly or serves to inhibit the production of a gonad-stimulating principle normally produced elsewhere in the body. On the latter hypothesis one possible explanation of the ovarian growth during the first six-day period in the gland-implanted animals is that during the two days elapsing between eyestalk (sinus gland) removal and the first implant, the blood titer of the inhibitor dropped to such a point that a gonad-promoting principle was permitted to be liberated into the blood. The first implant might be presumed to inhibit further production of the stimulating principle but not counteract the action of this factor already present.

An explanation based upon an hypothesis of a direct inhibition of ovarian growth is as follows: During the two days which elapsed between amputation of the eyestalks and the initial implantation of the sinus-gland tissue, there was a drop in titer of the inhibitory substance to an ineffectual level which permitted nearly as rapid growth in these ovaries as occurred in the destalked animals which received no implanted sinus glands. There may also be a delay in the production of an inhibiting concentration by the implants.

There is a suggestion in Table I that sinus-gland implants in the eyestalkless animals not only are able to inhibit growth in the partially developed ovaries, but may even effect a reduction in their size.

Summary

1. Removal of the eyestalks of adult females of *Uca pugilator* results in a period of rapid ovarian growth in which the increase in fresh weight of the gonad is approximately five-fold in a thirty-day period.

2. The period of ovarian growth is characterized by increase in oöcyte diameter and a color change from light pink to a deep purple-red.

3. Implantation of sinus-gland tissue into the abdomens of destalked females serves to inhibit to a large degree this rapid growth.

4. Six of the animals which had been deprived of their eyestalks laid mature eggs during the course of the experiment; none of the controls did so. Eggs produced by the experimental animals failed to become attached to the pleopods.

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